CHAPTER 3

THE ADDITIVE BURDEN OF IRON DEFICIENCY IN THE CARDIORENAL-ANEMIA AXIS: SCOPE OF A PROBLEM AND ITS CONSEQUENCES.

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European Journal of Heart Failure (2014) 16, 655–662

ABSTRACT

Aim

Iron deficiency (ID), anemia and chronic kidney disease (CKD) are common comorbidities in chronic heart failure (CHF) and all independent predictors of unfavorable outcome. The combination of anemia and CKD in CHF has been described as the cardiorenal-anemia syndrome. However, the role of ID within this complex interplay of co-existing pathologies is unclear.

Methods and results

We studied the clinical correlates of ID (defined as ferritin < 100 ug/L or 100–299 ug/L in combination with a transferrin saturation < 20%), anemia and renal dysfunction (defined as estimated glomerular filtration rate < 60 ml/min/1.73m2) and their prognostic implications in an international pooled cohort, comprising 1506 patients with CHF. Mean age was 64 ± 13 years, 74.2% was male and 47.3% had NYHA class III. The presence of ID, anemia, CKD or combination of these comorbidities was observed in 69.3% of the patients. During a median (Q1-Q3) follow-up of 1.92 years (1.18–3.26 years), 440 patients (29.2%) died. Eight-year survival rates decreased significantly from 58.0% for no comorbidities to 44.6%, 33.0% and 18.4%, for one, two or three comorbidities, respectively (p< 0.001). Multivariate hazard models revealed ID to be the key determinant of prognosis, either individually (p=0.042) or in combination with either anemia (p=0.006), CKD (p=0.029) or both (p=0.017).

Conclusions

Iron deficiency frequently overlaps with anemia and/or CKD in CHF. The presence of ID amplifies mortality risk, either alone or in combination with anemia, CKD or both, making it a potential viable therapeutic target.

ABBREVIATIONS

CHF	=	Chronic heart failure
CKD	=	Chronic kidney disease
CRAS	=	Cardiorenal-anemia syndrome
CRAIDS	=	Cardiorenal-anemia-iron deficiency syndrome
CRIDS	=	Cardiorenal-iron deficiency syndrome
hs-CRP	=	high-sensitive C-reactive protein
ID	=	Iron deficiency
IDA	=	Iron deficiency anemia
LVEF	=	Left ventricular ejection fraction
NT-proBNP	=	N-terminal pro-brain-type natriuretic peptide
NYHA	=	New York Heart Association
TSAT	=	Transferrin saturation

INTRODUCTION

Chronic heart failure (CHF) is best described as a systemic disease that also involves organs and tissues other than the heart. Anemia and chronic kidney disease (CKD) are two frequently observed comorbidities in CHF and both significantly influence morbidity and mortality.[1,2] In a large meta-analysis, anemia was present in 37% of all the CHF patients,[1] although incidence rates up to 60% have also been described.[3,4] Additionally, up to half of all patients with CHF have some form of renal dysfunction.[5] Progression of renal dysfunction and anemia can exacerbate CHF which, in turn, can cause more renal impairment and worsen anemia.[6] This vicious circle has been described by Silverberg *et al* as the cardiorenal-anemia syndrome (CRAS).[7]

Many factors may contribute to the development of CRAS and often more than one etiology is involved. Iron deficiency (ID) is part of the pathophysiology of anemia in both CHF and CKD,[8] which makes it an interesting treatment target in CRAS. Indeed, multiple studies have highlighted the potential clinical benefits of treating ID in anemic and nonanemic patients with CHF.[9–12] Additionally, ID with or without anemia is common in CHF, relates to disease severity and is itself an independent predictor of impaired exercise capacity, lower health-related quality of life and poorer prognosis.[13–15]

The pathophysiological importance of ID within this constellation of adverse phenomena is less well-described and its role in CHF patients with or without anemia and/or renal dysfunction merits further investigation and greater clinical awareness. To date, the presence and potential harmful effects of these partly overlapping pathologies have only been discussed in theory.[16] Does CRAS require additional terminology, like the cardiorenal-iron deficiency syndrome (CRIDS) or even the cardiorenal-anemia-iron deficiency syndrome (CRAIDS), to better characterize a patients' individual pathological condition and possible interventional requirements? The current study was initiated by a European iron consortium to provide evidence for and insight into these individual or combinations of comorbidities and their underlying prognostic consequences in a diverse CHF population.

METHODS

Component studies

The population of the present study consists of 5 cohorts from Poland, Spain and the Netherlands, comprising 1506 CHF patients with reduced or preserved left ventricular ejection fraction (LVEF), as previously described.[15]

Preserved left ventricular systolic function was defined as LVEF > 45%, as proposed in previous guidelines.17 Inclusion and exclusion criteria per participating study cohort are presented in *supplementary Table 1*. All study protocols were approved by local ethics committees and all patients gave separate written informed consent for this study. The study was conducted in accordance with the Declaration of Helsinki.

Pooled methodology

The pooled data in the current study were assessed at a patient level. All 5 cohorts, selected for analysis, had comparable clinical information available, including demographics, New York Heart Association (NYHA) classification, current medical therapy, physical examination, plasma and serum biochemistry results and LVEF (assessed via echocardiography or radionuclide ventriculography). None of the patients had received blood transfusions, erythropoietin therapy or intravenous iron therapy at the time of study entry. Vital status was determined via direct contact with patients or relatives or review of CHF clinical databases or hospital records. No patient was lost to follow-up and none received left ventricular assist device therapy during follow-up. The endpoint for the present study was all-cause mortality. Follow-up for survivors with events was censored when < 5% of the cohort was at risk (after 8 years).

Iron status and other laboratory measurements

Peripheral venous blood samples were collected from all patients. Hematologic indices were assessed in fresh venous blood using EDTA. After centrifugation, the remainder was frozen and stored prior to analysis. Anemia was defined as a hemoglobin level < 12 g/dL in women and < 13 g/dL in men.18 The following blood biomarkers reflecting iron status were measured: ferritin (µg/L), serum iron (umol/L), total iron binding capacity (µg/L) and transferrin (g/L). Transferrin measurements were available for most patients (n = 1202). Transferrin saturation (TSAT) was reported as serum iron/(25.2 x transferrin), multiplied by 100.19 When transferrin was not available (n = 304), TSAT was reported as a ratio of serum iron (µg/L) and TIBC (µg/L) multiplied by 100. There was a strong correlation between both TSAT measurements (R2 = 0.89, P < 0.001). Iron deficiency was defined as a ferritin level < 100 µg/L or serum ferritin 100–299 µg/L in combination with a TSAT < 20%. Similar definitions of ID have been used in recent observational and intervention trials in chronic HF.[12–14,20] Concentrations of N-terminal pro-brain-type natriuretic peptide (NT-proBNP, pg/mL) were measured using an immunoassay based on electrochemiluminescence on the Elecsys System

(Roche Diagnostics). Renal function was assessed estimating glomerular filtration rate (eGFR, mL/min/1.73m2) using the simplified Modification of Diet in Renal Disease (MDRD) equation. Chronic kidney disease was defined as an eGFR < 60 ml/min/1.73m2.21 Serum concentrations of high-sensitive C-reactive protein (hs-CRP, mg/L) were assessed at each institution using standard methods.

Statistical analyses

Data are expressed as means ± SD when normally distributed, as medians with lower and upper quartiles when non-normally distributed, or as numbers and percentages as categorical. Baseline characteristics were stratified by NYHA functional class. Inter-group differences were tested using the analysis of variance (ANOVA) test, Kruskal Wallis test or Pearson's $\chi 2$ test, when appropriate. For further analyses, skewed variables (NT-proBNP and hs-CRP) were transformed to a 2-log scale to achieve a normal distribution. This means that risk estimates should be interpreted as the relative risk if values were doubled (e.g. 2 to 4 mg/L).

Multiple logistic regression models were constructed to establish clinical determinants of iron deficiency anemia (IDA), CRIDS, CRAS and CRAIDS. All baseline variables with a significant univariable association with each individual syndrome ($p< 0.10$) were entered in a stepwise backward multivariate model based on the strength of their univariable association. Additional bootstrap resampling (1000 cycles) of the multivariate model was performed to validate the estimated model. Variables selected more than 700 times were assumed to be accurate.

Kaplan-Meier curves were constructed to demonstrate the impact per increasing number of comorbidities on cumulative survival. Differences in event-free survival rates were tested using the logrank test. Cox proportional hazard regression models were used to calculate the predictive value per increasing number of overlapping pathologies or individual (e.g. ID without anemia and CKD) and combination of comorbidities (e.g. IDA or CRIDS) for mortality. The proportionality assumption for the Cox regression analysis was evaluated using Schoenfeld residuals and was proven to hold for both analyses (chi-square 17.91; $P = 0.268$ and chi-square 19.88; $P = 0.402$, respectively). In two consecutive models adjustment was made for age, sex and a multivariate model including all variables with a significant univariable association. Reported probability values are two-sided and a p-value < 0.05 was considered statistically significant. All statistical models and analyses were performed using STATA version 11.0 (StataCorp LP, College Station, Texas, USA).

RESULTS

Baseline characteristics for all 1506 patients, stratified by NYHA functional class, are presented in *Table 1*. Overall, mean age was of 64 ± 13 years and 74.2% was male. Mean hemoglobin level was 13.6 ± 1.8 g/dL and mean eGFR was 79.9 ± 34.0 ml/min/1.73m2. Levels of iron status markers ferritin and TSAT were 154 ug/L (82 – 280) and 22.3% (14.5 – 32.7), respectively. Characteristics and individual or combined syndromes per participating cohort were also described (*Supplementary Table 2*).

Prevalence of individual and combined comorbidities

The prevalence of individual and overlap in comorbidities is displayed in *Figure 1*. Overall ID, anemia, or CKD was present in 50.0%, 28.3% and 28.4% respectively, with a global prevalence of 69.3% for at least one of these comorbidities. The prevalence of combined comorbidities rose with increasing NYHA class (*Figure 2*). Iron deficiency was more common in patients with versus without CKD (56.4% vs. 47.4%, $P = 0.002$), and in patients with versus without anemia (61.2% vs. 45.6%, $p< 0.001$). Stratification by quartiles of hemoglobin and renal function (expressed as eGFR) showed an increase in ID with decreasing hemoglobin levels and worse renal function (*Figure 3*). Even in the highest quartile of both hemoglobin and eGFR, the prevalence of ID still was above 30%. The presence of diabetes was more common if patients with IDA, CRAS or CRAIDS (with versus without; all $P < 0.01$), but not for CRIDS or lone comorbidities (with versus without; all $P > 0.05$).

Clinical determinants of combined syndromes in chronic heart failure.

Multivariable logistic regression analyses are described in *Table 2*. Both age and hs-CRP had a strong positive association with all combinations of syndromes. In addition, hs-CRP was not associated with lone comorbidities (all $P > 0.05$), suggesting an inflammatory background when a combination of comorbidities is present. Hemoglobin was a powerful predictor for CRIDS, whereas renal function was strongly predictive for IDA, and TSAT-but not ferritin levels-for CRAS. Furthermore, levels of NT-proBNP were only associated with a combined syndrome when renal dysfunction was involved, whereas mean corpuscular volume was only associated with a combined syndrome if patients were iron deficient. In bootstrap analyses, these variables remained highly selected for each syndrome. Interestingly, diabetes was only associated with the presence of IDA, CRAS or CRAIDS-and not CRIDS-in univariable regression analyses (*Supplementary Table 3*). However, this significance was lost in multivariate analyses.

Table 1. Baseline characteristics stratified by New York Heart Association functional class.

Variables	All patients	NYHA class I	NYHA class II	NYHA class III	NYHA class IV	P - value
	n = 1506	n = 121	n = 577	n = 712	n = 96	
Age (years)	64 ± 13	58 ± 14	62 ± 13	66 ± 13	67 ± 15	< 0.001
Men (%)	74.2	88.4	77.3	70.4	65.6	< 0.001
BMI (kg/m2)	27.1 ± 5.9	27.7 ± 5.6	27.5 ± 4.9	26.7 ± 5.9	25.0 ± 7.3	< 0.001
Ischemic etiology (%)	60.2	62.8	60.3	59.7	60.4	0.935
LVEF (%)	33 ± 14	35 ± 12	34 ± 13	32 ± 14	28 ± 13	< 0.001
HFrEF (%)	87.3	87.6	87.4	87.0	88.5	0.973
Comorbidities (%)						
ID alone1	23.5	26.5	26.3	21.5	17.7	0.047
Anemia alone2	7.0	5.0	6.9	7.3	7.3	0.827
CKD alone3	8.4	5.0	6.1	10.4	10.5	0.013
IDA	10.5	7.9	8.3	12.2	15.6	0.023
CRAS	4.0	4.1	2.8	4.6	6.3	0.231
CRIDS	9.2	2.3	4.5	9.4	14.0	< 0.001
CRAIDS	6.8	2.5	4.2	8.3	17.7	< 0.001
Diabetes mellitus	34.9	32.2	27.2	39.0	54.2	< 0.001
AF	19.7	15.7	17.9	22.2	16.7	0.120
Hypertension	20.3	31.4	20.8	19.4	10.4	0.001
Laboratory measurements						
Hemoglobin (g/dL)	13.6 ± 1.8	14.0 ± 1.5	13.8 ± 1.7	13.4 ± 1.9	13.0 ± 2.2	0.001
MCV (fl)4	90.9 ± 5.9	90.1 ± 5.7	91.1 ± 5.5	90.8 ± 6.0	91.0 ± 6.9	0.441
Serum iron (µg/L)	73 (49 – 105)	93 (66 – 121)	86 (58 – 118)	64 (42 – 94)	60 (42 – 94)	< 0.001
Ferritin (µg/L)	154 (82 – 280)	173 (87 – 278)	164 (91 – 296)	149 (79 – 267)	136 (74 – 242)	0.055
TSAT (%)	22.3 (14.5 – 32.7)	27.3 (19.3 – 40.2)	25.8 (17.6 – 36.6)	19.1 (17.6 – 28.9)	18.0 (12.0 – 27.5)	< 0.001

Table 1. Baseline characteristics stratified by New York Heart Association functional class. (continued)

Variables	All patients	NYHA class I	NYHA class II	NYHA class III	NYHA class IV	P - value
	n = 1506	n = 121	n = 577	n = 712	n = 96	
hs-CRP (mg/L)5	2.9 (1.3 – 6.9)	1.6 (1.1 – 4.7)	2.0 (1.2 – 4.9)	3.8 (1.6 – 9.0)	5.1 (1.9 – 11.0)	< 0.001
NT-proBNP (pg/mL)	1395 (550 – 3572)	606 (233 – 1622)	963 (431 – 2184)	1869 (853 – 4402)	4094 (1426 – 9684)	< 0.001
Sodium (mmol/L)	139.1 ± 5.4	140.7 ± 3.0	140.0 ± 3.4	138.4 ± 6.7	137.1 ± 5.4	0.013
eGFR (ml/min/1.73m2)	79.9 ± 34.2	92.5 ± 35.0	86.4 ± 31.9	74.0 ± 33.9	70.1 ± 34.2	< 0.001
Concomitant treatment (%)						
ACE inhibitor and/or ARB	90.9	95.0	92.6	90.7	77.1	< 0.001
Beta blocker	89.9	97.5	95.3	89.6	84.3	< 0.001
Loop diuretic	79.2	47.9	70.0	90.5	90.6	< 0.001
MRA	50.1	34.9	46.9	55.6	56.8	< 0.001
Statin	64.0	71.9	70.9	57.6	60.4	< 0.001
Antiplatelet or anticoagulant	84.0	79.3	84.2	85.3	79.2	0.214

Values are means (standard deviation), medians (interquartile range), or proportions (%).
Abbreviations: BMI = body mass index; CKD = chronic kidney disease; CRAS = cardiorenal–anemia syndrome; CRAIDS = cardiorenal–anemia–iron deficiency syndrome; CRIDS = cardio-renal–iron deficiency syndrome; eGFR = estimated glomerular filtration rate = HF, heart failure; HFrEF = heart failure with reduced ejection fraction; hs-CRP = high sensitivity C-reactive protein; ID = iron deficiency; IDA = iron deficiency anemia; MCV = mean corpuscular volume; MRA = mineralocorticoid receptor antagonist; TSAT = transferrin saturation.
1 ID was defined as ferritin < 100 µg/L or 100–299 µg/L with a TSAT < 20%.
2 Anemia was defined as hemoglobin < 12 g/dL in women and < 13 g/dL in men.
3 CKD was defined as an eGFR < 60 mL/min/1.73 m2.
4 MCV was measured in 1123 patients.
5 hs-CRP was measured in 1000 patients

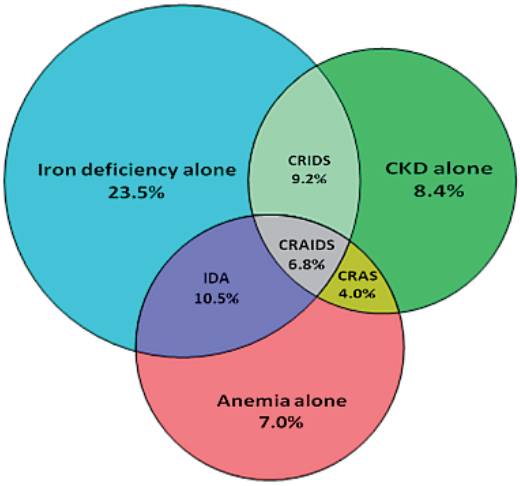

Figure 1. Venn diagram showing proportional prevalence of individual or combined syndromes in chronic heart failure

Association of comorbidities with prognosis in chronic heart failure

During a median follow-up of 1.92 years (1.18 – 3.26 years), 440 patients (29.2%) died. No significant association was observed between study cohort and outcome (P = 0.78). Similarly, interaction analyses revealed no significant association between study cohort and ID (P = 0.62), anemia (P = 0.18), or CKD (P = 0.51). Eight year event-free survival rates significantly decreased from 58.0% (95% confidence interval [CI] 42.6–70.6%), to 44.6% (95%CI 35.8–53.2%), 33.0% (95%CI 25.2–41.1%) and 18.4% (95%CI 9.0–30.3%) when the number of present comorbidities increased from no comorbidity to all three comorbidities together (log rank *P* < 0.001) (*Figure 4*). In consecutive hazard regression models, the risk for mortality significantly enhanced per increasing number of comorbidities (*Table 3A*). Each individual or combination of comorbidities also predicted poor survival (*Table 3B*). Subsequent multivariate analyses revealed ID to remain independently associated with an increased mortality risk, either alone or in combination with either CKD, anemia, or both (*Table 3B*).

DISCUSSION

In this large pooled cohort of diverse CHF patients, ID is common and fre-

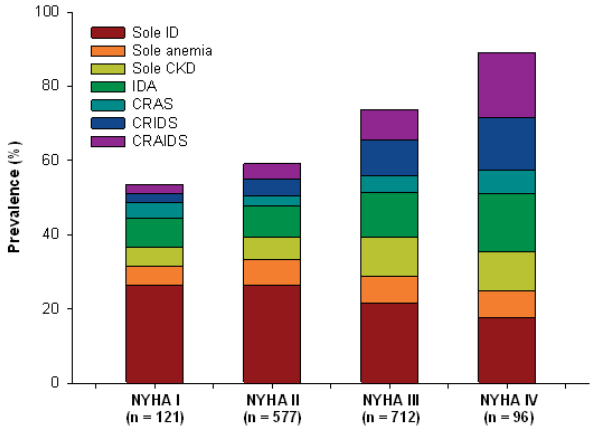

Figure 2. Prevalence of individual or overlapping comorbidities, stratified by NYHA functional class.

quently overlaps with anemia, CKD or both. The prevalence of ID increases with worse renal function and/or hemoglobin levels. Finally, the presence of ID alone or in combination with anemia, CKD, or both was independently associated with an increased risk for death.

Prevalence of individual or combined comorbidities

Both CKD and anemia in HF have received a great deal of attention in recent decades. Renal dysfunction is often involved in CHF since both organs have an interdependent role in blood pressure control and blood volume homeostasis. Another common comorbidity in CHF is anemia, its prevalence increasing with the severity of concomitant renal impairment.22 In turn, anemia can worsen CHF and CKD leading to the vicious circle called CRAS.7 Scrutinio *et al* found that CRAS was present in 21.1% of 951 patients with systolic HF,22 whereas a prevalence of 29.9% in 748 HF patients was reported by Lu *et al*.23 In the present study, we found a prevalence of 4.0% for CRAS. This lower prevalence could be partially explained by the fact that we did not include the presence of ID in the definition of CRAS.

In recent years, more research has been focusing on the prevalence and prognostic role of ID in patients with CHF. However, since there is no clear-cut definition for ID in patients with HF, a wide variation in prevalence has been reported.[15,20,24] One study, which used the gold standard of bone marrow iron staining, found that 73% of patients with advanced HF and anemia had depleted iron stores.25 Nonetheless, the criteria most commonly used and implemented in the most recent HF guidelines of the European

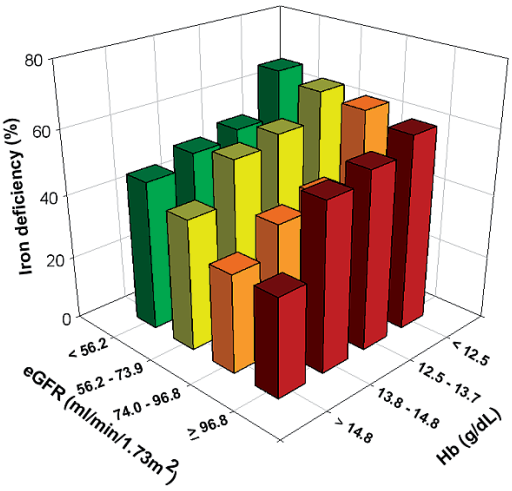

Figure 3 Prevalence of iron deficiency according to quartiles of renal function and levels of hemoglobin.

Society of Cardiology are a ferritin level < 100 µg/L or ferritin 100 – 299 µg/L in combination with a TSAT < 20%.17 Using this definition, we demonstrated that the prevalence of ID alone, IDA, CRIDS or CRAIDS was 23.5%, 10.5%, 9.2% and 6.8% respectively.

Predictors of combined comorbidities

Both increasing age and higher hs-CRP levels were independently correlated with all combined syndromes. Older age is a well-known risk factor for ID, anemia or CKD in CHF. Additionally, the positive association of hs-CRP levels with all combined syndromes suggests that the underlying pathophysiological interplay between ID, anemia and CKD may have an inflammatory origin. Furthermore, hs-CRP was not associated with any lone comorbidity in this study, supporting this inflammatory hypothesis when only a combination of comorbidities is present. Indeed, CHF patients often present with a low-grade generalized inflammatory status which is characterized by trapping of iron within the cells of the reticuloendothelial system leading to functional ID, a major component of anemia of chronic disease.26 We also observed a significant univariable relationship between diabetes and all combined syndromes (except for CRIDS), though this significance was lost in multivariable analyses. It has been suggested that diabetics are more prone to develop both anemia and renal dysfunction compared to non-diabetics. However, only iron overload has been associated with dia-

Table 2. Clinical associates of combined syndromes in patients with chronic heart failure.

Variables	IDA		CRIDS		CRAS		CRAIDS	
	OR (95% CI)	p - value	OR (95% CI)	P - value	OR (95% CI)	P - value	OR (95% CI)	P - value
Age (per 5 years)	1.30 (1.10 – 1.53)	0.002	1.25 (1.07 – 1.46)	0.005	1.64 (1.41 – 2.83)	< 0.001	1.44 (1.13 – 1.86)	0.003
Hemoglobin (per 1 g/dL)	NA	NA	0.73 (0.48 – 0.92)	0.001	NA	NA	NA	NA
MCV (per 1 fl)1	0.91 (0.86 – 0.97)	0.002	0.93 (0.87 – 0.98)	0.010	-	-	0.88 (0.80 – 0.96)	0.005
TSAT (per 5%)	NA	NA	NA	NA	0.63 (0.49 – 0.82)	< 0.001	NA	NA
eGFR (per 5 ml/min/1.73m2)	0.85 (0.76 – 0.91)	< 0.001	NA	NA	NA	NA	NA	NA
hs-CRP (per doubling)2	1.28 (1.07 – 1.55)	0.010	1.24 (1.04 – 1.46)	0.011	1.17 (1.04 – 1.32)	0.008	1.43 (1.10 – 1.87)	0.008
NT-proBNP (per doubling)	-	-	1.65 (1.33 – 2.05)	< 0.001	1.45 (1.07 – 1.98)	0.017	1.51 (1.11 – 1.76)	< 0.001

Values are odds ratios ± 95% confidence intervals.1Mean corpuscular volume was measured in 1123 patients.2High-sensitive C-reactive protein was measured in 1000 patients.
Abbreviations: CI = Confidence interval; NA = Not applicable; OR = Odds ratio; other abbreviations as in Table 1.

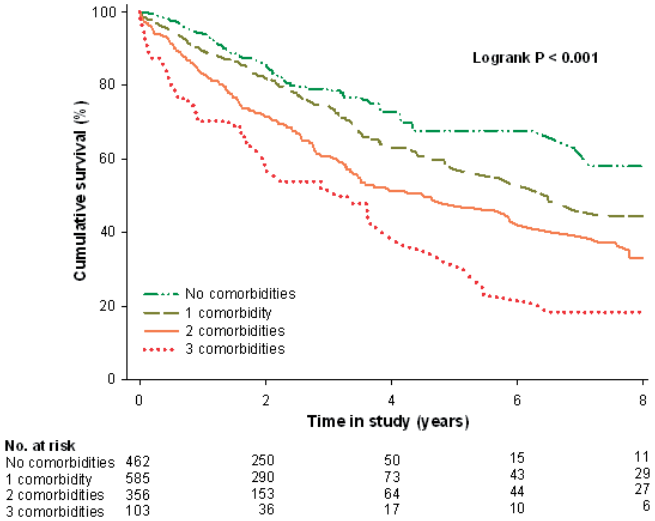

Figure 4. Kaplan–Meier curves depicting event-free survival rates with an increasing number of co-morbidities in patients with chronic heart failure.

betes,27 which does not explain the observed association with IDA. The role of diabetes in the presence of ID, anemia or CKD remains to be elucidated.

Prognosis and clinical implications

Both CKD and anemia have been extensively described as prognostic risk factors in CHF.1,2 However, only a few studies have reported on ID as an independent outcome predictor.[15,20,24] In this study, we demonstrate for the first time that ID, alone or in combination with anemia, CKD or both, is associated with increased mortality. Our findings and recent literature suggest that the currently used term CRAS may neglect the clinical and prognostic importance of ID in CHF, both within and mostly beyond the process of erythropoiesis. Besides its clinical consequences directly related to erythropoiesis, iron plays an important role in oxygen storage (in myoglobin) and oxygen metabolism in skeletal and heart muscle (in oxidative enzymes and respiratory chains proteins). Even in nonanemic patients, ID has been associated with decreased aerobic performance and exercise intolerance.28 Moreover, structural abnormalities in cardiac myocytes have also been reported.29 Therefore, maintaining normal iron metabolism is crucial, especially for cells of high mitogenic potential (e.g. hematopoietic cells) or high energy demand (e.g. skeletal myocytes, cardiomyocytes).

Table 3. Age, sex, and multivariate-adjusted Cox regression per increasing number of co-morbidities or comorbidity-specific analysis for all-cause mortality.

Variable	Age- and sex-adjusted			Multivariable[1]		
	HR (95% CI)	Z	P - value	HR (95% CI)	Z	P - value
Number of comorbidities						
1 comorbidity	1.34 (1.03 – 1.76)	2.14	0.032	1.31 (0.98 – 1.77)	1.82	0.069
2 comorbidities	2.04 (1.54 – 2.73)	4.90	< 0.001	1.62 (1.16 – 2.26)	2.66	0.008
3 comorbidities	3.12 (2.18 – 4.44)	6.27	< 0.001	1.83 (1.17 – 2.85)	2.83	0.005
Individual comorbidities						
ID alone	1.49 (1.03 – 1.83)	1.65	0.038	1.42 (1.02 – 1.98)	2.04	0.042
Anemia alone	1.95 (1.27 – 2.98)	3.07	0.002	1.42 (0.83 – 2.42)	1.27	0.203
CKD alone	1.58 (1.05 – 1.95)	1.99	0.024	1.09 (0.73 – 1.64)	0.44	0.663
IDA	1.86 (1.28 – 2.70)	3.26	0.001	1.88 (1.20 – 2.94)	2.75	0.006
CRAS	2.45 (1.60 – 3.77)	4.10	< 0.001	1.21 (0.70 – 2.09)	0.67	0.500
CRIDS	2.07 (1.47 – 2.92)	4.16	< 0.001	1.55 (1.04 – 2.30)	2.18	0.029
CRAIDS	3.15 (2.20 – 4.49)	6.31	< 0.001	1.73 (1.11 – 2.72)	2.40	0.017

Values are hazard ratios ± 95% confidence intervals.
Abbreviations: CI = Confidence interval; HR = Hazard ratio; other abbreviations as in Table 1.
1Multivariable model is adjusted for all univariate significant variables (age, sex, body mass index, LVEF, NYHA functional class, presence of diabetes, levels of sodium, NT-proBNP and hs-CRP, treatment with angiotensin-converting enzyme inhibitors and/or angiotensin receptor blockers, statins, loop diuretics and study center).

The role of ID in CHF patients, with or without anemia and/or CKD, therefore merits clinical awareness, and more insight into the pathophysiology and interplay between ID, anemia and CKD is warranted. If patients with HF, renal dysfunction, and ID were to show clinical improvements, even in the absence of anemia, the acronym CRIDS might possibly be introduced into the clinical field. Indeed, a recent substudy of the FAIR-HF trial showed similar results in both anemic and non-anemic patients treated with ferric carboxymaltose on both primary and secondary study endpoints.30 However, confirmation of these findings from ongoing and future clinical trials are warranted to determine whether more accurate terminology, like CRIDS, is needed to describe specific combinations of these adverse phenomena and their possible treatment requirements. In addition, despite the association of ID with prognosis, observed in this and previous studies, to date there is no evidence about the effects of iron replacement therapy on patients' survival in patients with HF.

Limitations

First, the present cohort study was retrospective. However, all data was assessed at a patient level, no patient was lost to follow-up and the dura-

tion of follow-up makes it relevant to clinicians. Our study also complies with the guidelines for observational studies.31 Second, the present study only collected data from a single time point, so cannot comment on the effects of changes in iron status, hemoglobin or renal function over time. More studies with serial measurements over time are warranted. Third, no follow-up information was available regarding treatment of deficiencies or device therapy (except for left ventricular assist device therapy). In addition, despite that none of the patients received blood transfusions, erythropoietin therapy or intravenous iron therapy at the time of study entry, we do not have an exact timescale of all patients, regarding these treatments, that precluded them from the current analysis. Fourth, red cell distribution width (RDW) was not measured in this study. It has been suggested that RDW reflects ID, anemia and renal dysfunction and is an independent predictor of mortality.32 Furthermore, no information on hospitalizations (cardiovascular or HF), heart transplantation, or cause of death was available for the present analysis. More studies on ID, anemia, CKD and combination of these comorbidities and their association with cardiovascular and HF hospitalizations or mortality are needed. Finally, accurate diagnosis of ID in chronic HF is mandatory. Although current definitions of ID, based on blood markers, may be unreliable, using the gold standard (bone marrow iron staining) in every CHF patient suspected of ID is unrealistic. Patients with CHF, CKD or both present with a generalized inflammatory status and activation and production of inflammatory cytokines and acute phase proteins, such as ferritin. Therefore, it might be better to use a higher cutoff for ferritin to define ID in patients with CHF or CKD.33,34 Nevertheless, more studies are needed to identify potential new or additional serum markers reflecting iron status and compare them with the gold standard of bone marrow iron staining.

CONCLUSIONS

Iron deficiency, either alone or in overlap with anemia, CKD or both, is common in CHF and its prevalence increases with worse renal function and lower hemoglobin levels. The presence of overlapping comorbidities increases with disease severity. In this study, ID is a key determinant of prognosis, either alone or in combination with anemia, CKD or both, making it a potential therapeutic target in these high risk patients.

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SUPPLEMENTAL FILES

Table S1. Inclusion and exclusion criteria within study cohorts.

All cohorts N = 1506	Inclusion criteria	Exclusion criteria
Dutch cohort 1 N = 202 (27)	1. NYHA class III-IV.	1. Dementia or psychiatric illness
	2. Stable HF in conjunction with echocardiographic findings of a reduced left ventricular systolic function (LVEF ≤ 45%) or preserved left ventricular systolic function.	2. Staying in a nursing home
	3. Able to understand the study procedures and willing to provide informed consent.	3. Other disease with expected survival < 1 year.
		4. Participation in other trial(s)
		5. Ongoing or planned hospitalization
		6. Undergoing kidney replacement therapy
Dutch cohort 2 N = 9526	1. NYHA class II-IV	1. History of myocardial infarction or stroke in previous 6 months.
	2. Echocardiographic LVEF ≤ 45%.	2. Severe valvular dysfunction.
	3. HF duration of at least 3 month.	3. Severe pulmonary disease or uncontrolled diabetes.
	4. Stable HF medical therapy for at least 1 month.	4. History of systemic inflammatory or collagen vascular disease.
	5. Able to understand the study procedures and willing to provide informed consent.	5. Active and/or treated malignancies within 12 months before inclusion.
		6. Clinically significant renal dysfunction or liver function abnormalities.
		7. Severe anemia at baseline (hemoglobin < 10 g/dL).
		8. Pregnancy or active breast-feeding (pregnancy tests will be performed on all female subjects of child-bearing potential)
		9. Use of any investigational drugs (within 30 d before screening).

Table S1. Inclusion and exclusion criteria within study cohorts. (continued)

All cohorts N = 1506	**Inclusion criteria**	**Exclusion criteria**
Polish cohorts N = 7351315	1. NYHA class I-IV.	1. Acute coronary syndrome, coronary revascularization or any major surgery within 3 months preceding the study
	2. A documented history of HF of ≥ 6 months.	2. Unplanned hospitalization due to heart failure deterioration or any other cardiovascular reason within 1 month preceding the study
	3. Left ventricular ejection fraction ≤ 45% as assessed by echocardiography.	3. Any acute or chronic illness that might influence iron metabolism.
	4. Clinical stability and unchanged medications for ≥ 1 month preceding the study.	4. Any anemia or/and iron deficiency treatment either at the time of the study or within the past 12 months.
	5. Able to understand the study procedures and willing to provide informed consent.	
Spanish cohort N = 47425	1. NYHA class I-IV.	1. Significant primary valvular disease or significant pericardial disease.
	2. Clinically stable condition ≥ 1 month preceding the study.	2. Severe anemia (hemoglobin < 8.5 g/dL).
	3. A reduced left ventricular systolic function (LVEF ≤ 45%) or preserved left ventricular systolic function.	3. Restrictive and hypertrophic cardiomyopathy.
	4. Patients able to understand the study procedures and willing to provide informed consent.	4. Active malignancy, presence of an active infection or clinically significant liver function abnormalities.

Abbreviations: NYHA = New York Heart Association, LVEF = Left ventricular ejection fraction.

Table S2. Baseline characteristics stratified by study cohort.

Variables	All patients	Holland 1	Holland 2	Poland 1	Poland 2	Spain	P - value
	N = 1506	N = 202	N = 95	N = 364	N = 371	N = 474	
Age (years)	64 ± 13	71 ± 12	60 ± 12	61 ± 11	54 ± 10	72 ± 11	< 0.001
Men (%)	74.2	73.3	78.9	82.7	86.8	57.2	< 0.001
BMI (kg/m2)	27.5 ± 4.8	26.3 ± 5.6	27.9 ± 4.2	27.8 ± 4.2	26.5 ± 4.2	27.0 ± 6.7	0.072
Ischemic cause (%)	60.2	61.9	69.5	70.3	70.9	41.6	< 0.001
LVEF (%)	33 ± 14	31 ± 9	32 ± 9	31 ± 9	24 ± 6	42 ± 17	< 0.001
HFrEF (%)	87.3	96.5	100	100	100	61.0	< 0.001
NYHA functional class (%)							< 0.001
I/II	46.3	0.0	64.2	66.2	47.2	46.6	
III	47.3	96.5	32.6	31.0	43.6	44.5	
IV	6.4	3.5	3.2	2.8	9.2	8.9	
Comorbidities (%)							
ID alone1	23.5	15.8	56.8	19.5	20.8	25.3	< 0.001
Anemia alone2	7.0	1.5	4.2	3.3	8.1	11.8	< 0.001
CKD alone3	8.4	18.8	3.2	9.3	10.0	3.0	< 0.001
IDA	10.5	3.5	2.1	5.2	7.3	21.7	< 0.001
CRAS	4.0	5.9	0.0	5.2	1.6	4.9	0.008
CRIDS	9.2	30.2	5.3	8.8	5.4	4.2	< 0.001
CRAIDS	6.8	15.8	2.1	5.2	1.4	9.5	< 0.001
Diabetes mellitus	34.9	29.2	16.8	34.3	26.4	48.1	< 0.001
AF	19.7	28.7	0.0	25.3	0	30.8	< 0.001
Hypertension	20.3	26.2	11.6	25.6	8.4	24.9	< 0.001

Table S2. Baseline characteristics stratified by study cohort. (continued)

Variables	All patients	Holland 1	Holland 2	Poland 1	Poland 2	Spain	P - value
	N = 1506	N = 202	N = 95	N = 364	N = 371	N = 474	
Laboratory measurements							
Hb (g/dL)	13.6 ± 1.8	13.6 ± 1.6	14.4 ± 1.2	14.0 ± 151	14.2 ± 1.6	11.3 ± 1.1	< 0.001
MCV (fL)4	90.9 ± 5.9	NA	NA	90.7 ± 1.5	91.0 ± 7.6	88.4 ± 6.0	NA
Serum iron (ug/L)	73 (49 – 105)	100 (82 – 131)	100 (82 – 131)	59 (42 – 84)	74 (54 – 103)	45 (32 – 61)	< 0.001
Ferritin (ug/L)	154 (82 – 280)	140 (74 – 272)	127 (71 – 203)	164 (87 – 278)	179 (102 – 310)	145 (75 – 274)	< 0.001
TSAT (%)	22.3 (14.5 – 32.7)	14.3 (6.5 – 22.0)	17.6 (14.0 – 22.0)	31.1 (21.4 – 42.2)	29.3 (20.2 – 39.6)	17.7 (12.0 – 24.9)	< 0.001
NT-proBNP (pg/mL)	1395 (550 – 3572)	2135 (989 – 4473)	388 (143 – 807)	1467 (488 – 3951)	1364 (652 – 3109)	1395 (652 – 3109)	< 0.001
Serum sodium (mmol/L)	139 ± 5	138 ± 3	140 ± 2	141 ± 3	136 ± 4	140 ± 7	< 0.001
hs-CRP (mg/L)5	2.9 (1.3 – 6.9)	5.0 (2.0 – 14.0)	1.6 (0.8 – 3.7)	3.1 (1.4 – 6.8)	2.4 (1.2 – 5.6)	NA	NA
eGFR (ml/min/1.73m2)	79.9 ± 33.8	51.1 ± 14.1	79.9 ± 20.3	71.0 ± 20.4	84.0 ± 25.8	97.8 ± 45.6	< 0.001
Treatment (%)							
ACE inhibitor and/or ARB	90.9	95.1	94.7	94.2	94.6	82.9	< 0.001
Beta blocker	89.9	62.4	93.7	96.2	98.9	89.0	< 0.001
Loop diuretic	79.2	97.0	55.8	54.4	86.0	90.1	< 0.001
Statin	64.0	39.6	81.1	78.3	71.4	54.2	< 0.001
MRA	47.5	0.0	29.5	33.8	91.6	47.5	< 0.001
Antiplatelet and/or anticoagulant	84.0	89.6	75.8	84.9	83.0	83.3	0.078

Values are means ± standard deviation, medians (interquartile range) or proportions (%).1ID was defined as ferritin < 100 μg/L or 100–299 μg/L with a TSAT < 20%.2Anemia was defined as hemoglobin < 12 g/dL in women and < 13 g/dL in men.[3]CKD was defined as an eGFR < 60 ml/min/1.73m2. 4MCV was measured in 1123 patients.[5]hs-CRP was measured in 1000 patients. For abbreviations, see Table 1.

Table S3. Univariable association of clinical variables with combined syndromes in patients with chronic HF.

Variables	IDA		CRIDS		CRAS		CRAIDS	
	OR (95% CI)	p - value	OR (95% CI)	p - value	OR (95% CI)	p-value	OR (95% CI)	p-value
Age (per 5 years)	1.14 (1.07 – 1.22)	< 0.001	1.20 (1.11 – 1.29)	< 0.001	1.40 (1.34 – 1.58)	< 0.001	1.46 (1.32 – 1.61)	< 0.001
Female sex (yes vs. no)	1.16 (0.81 – 1.68)	0.421	2.19 (1.53 – 3.15)	< 0.001	1.35 (0.77 – 2.35)	0.293	1.39 (0.78 – 3.03)	0.488
BMI (per kg/m2)	0.99 (0.96 – 1.02)	0.546	0.99 (0.97 – 1.02)	0.692	0.95 (0.92 – 0.98)	0.004	1.00 (0.97 – 1.04)	0.885
Ischemic HF etiology (yes vs. no)	0.91 (0.65 – 1.27)	0.588	1.14 (0.79 – 1.64)	0.478	1.86 (1.04 – 3.32)	0.037	1.24 (0.82 – 1.90)	0.301
LVEF (per %)	1.02 (1.01 – 1.04)	< 0.001	0.98 (0.97 – 0.99)	0.011	1.00 (0.98 – 1.02)	0.900	1.02 (1.01 – 1.04)	< 0.001
NYHA class								
III vs. I/II	1.60 (1.12 – 2.27)	0.010	3.76 (2.46 – 5.78)	< 0.001	1.57 (0.90 – 2.73)	0.114	2.24 (1.41–3.59)	0.001
IV vs. I/II	2.12 (1.14 – 3.92)	0.016	2.38 (1.09 – 5.21)	0.029	2.15 (0.85 – 5.46)	0.108	5.35 (2.80 – 10.2)	< 0.001
Diabetes (yes vs. no)	1.69 (1.22 – 2.37)	0.002	0.77 (0.52 – 1.13)	0.178	1.91 (1.14 – 3.21)	0.014	2.26 (1.51 – 3.39)	< 0.001
AF (yes vs. no)	1.14 (0.76 – 1.70)	0.533	1.38 (0.92 – 2.08)	0.124	0.92 (0.47 – 1.78)	0.793	1.34 (0.84 – 2.14)	0.223
Hypertension (yes vs. no)	0.95 (0.63 – 1.44)	0.818	1.21 (0.80 – 1.83)	0.380	0.68 (0.33 – 1.40)	0.299	1.28 (0.80 – 2.04)	0.303
Hemoglobin (per 1 g/dL)	NA	NA	0.81 (0.69 – 0.93)	< 0.001	NA	NA	NA	NA
MCV (per 1 fL)1	0.93 (0.90 – 0.96)	< 0.001	0.96 (0.92 – 0.99)	0.040	1.01 (0.96 – 1.06)	0.774	0.91 (0.87 – 0.95)	< 0.001
Ferritin (per doubling)	NA	NA	NA	NA	2.12 (1.96 – 3.22)	< 0.001	NA	NA
TSAT (per 5%)	NA	NA	NA	NA	0.66 (0.41 – 0.87)	< 0.001	NA	NA
hs-CRP (per doubling)2	1.16 (1.01 – 1.35)	0.045	1.23 (1.11 – 1.37)	< 0.001	1.27 (1.06 – 1.53)	0.009	1.43 (1.23 – 1.65)	< 0.001
NT-proBNP (per doubling)	1.11 (1.02 – 1.21)	0.020	1.35 (1.22 – 1.49)	< 0.001	1.77 (1.51 – 2.08)	< 0.001	1.37 (1.23 – 1.53)	< 0.001
Sodium (per mmol/L)	1.05 (1.01 – 1.10)	0.018	0.99 (0.97 – 1.02)	0.472	0.99 (0.96 – 1.03)	0.783	0.99 (0.96 – 1.03)	0.747
eGFR (per 5 ml/min/1.73m2)	0.93 (0.92 – 0.95)	< 0.001	NA	NA	NA	NA	NA	NA
Treatment with ACE inhibitor and/or ARB (yes vs. no)	0.66 (0.40 – 1.09)	0.102	0.74 (0.43 – 1.29)	0.286	0.31 (0.16 – 0.57)	< 0.001	0.27 (0.17 – 0.44)	< 0.001
Treatment with beta blocker (yes vs. no)	0.75 (0.45 – 1.24)	0.259	0.46 (0.29 – 0.74)	0.001	1.01 (0.43 – 2.39)	0.981	0.50 (0.29 – 0.85)	0.011

Table S3. Univariable association of clinical variables with combined syndromes in patients with chronic HF. (continued)

Variables	IDA		CRIDS		CRAS		CRAIDS	
	OR (95% CI)	p - value	OR (95% CI)	p - value	OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment with loop diuretics (yes vs. no)	2.50 (1.47 – 4.27)	0.001	2.96 (1.62 – 5.42)	< 0.001	0.95 (0.71 – 1.77)	0.863	2.29 (1.21 – 4.34)	0.011
Treatment with MRA (yes vs. no)	1.15 (0.88 – 1.52)	0.307	0.78 (0.56 – 1.09)	0.146	0.53 (0.33 – 0.84)	0.008	0.54 (0.37 – 0.77)	0.001
Treatment with statin (yes vs. no)	0.78 (0.60 – 1.09)	0.155	0.60 (0.43 – 0.86)	0.005	1.05 (0.61 – 1.80)	0.871	0.84 (0.56 – 1.26)	0.404
Treatment with antiplatelet and or anticoagulant (yes vs. no)	1.02 (0.65 – 1.59)	0.948	1.00 (0.61 – 1.62)	0.984	1.75 (0.75 – 4.11)	0.201	1.04 (0.60 – 1.80)	0.893

Values are odds ratios ± 95% confidence intervals.1Mean corpuscular volume was measured in 1123 patients.2High-sensitive C-reactive protein was measured in 1000 patients. Abbreviations: CI = Confidence interval; NA = Not applicable; OR = Odds ratio; other abbreviations as in Table 1.

IRON STATUS, HEMOGLOBIN LEVELS AND NEW ONSET HEART FAILURE IN THE GENERAL POPULATION

II